SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract No. NS 5-2322

Quarterly Progress Report #7

October 31, 1996

The University of Michigan

David C. Martin and K. Sue O'Shea

Quarterly Progress to: National Institute of Health

Contract Monitor: William Heetderks, Ph.D.

Research Contract "Surface Modification for Biocompatibility"

Contract No. NS 5-2322

Principal Investigators: David C. Martin and K. Sue O'Shea

Date: October 31, 1996

Overview

This report is a summary of our activity in the seventh quarter of the contract, corresponding to the third quarter of 1996. This report provides an overview of the major results to date and discusses our plans for the future. As before, we have been working to evaluate (1) protein polymer film deposition and morphology, (2) bioactivity of protein polymer films *in vitro*, and (3) bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition, Morphology, and Device Characterization

Progress:

We have now completed a study of the incorporation of small molecules into protein polymers and aliphatic poly(amides) (nylon 6,6). The addition of these secondary components to the solution does not impede the processing of the material into fibers and filaments via the electrostatic deposition process.

Molecules studied include salicylic acid (aspirin), 138.1 g/mol, amo~~o~~xicillin (365.4 g/mol), bacitracin (1420.1 g/mol), caffeine (194 g/mol), cephalospoin C (415.4 g/mol), erythromycin (733.9 g/mol), hygromycin B (527.5 g/mol), kanamycin (484.64 g/mol), neomycin sulfate (908.9 g/mol), nystatin (926.1 g/mol), and tetracycline (444.4 g/mol).

The microstructure of the filaments are a function of the amount of added drug. In general, as the amount of drug increases, there is a transition from a filamentous texture to a more irregular drop-like texture. Studies of film morphology as a function of time (in water) show irregularities in surface texture consistent with the leaching of the small molecules out of the film. An in-vivo test of the efficacy of antibiotic release from the films was pursued and proved to be succesful, as discussed in the following section.

These results confirm the flexibility of blending secondary components into the protein polymer films with little or no variation in our processing methodologies. Further studies are necessary to tailor the release rates appropriate for a given application. It is reasonable to anticipate that we will be able to control the release rate by variations in the filament diameter.

We have now begun to correlate the variations in surface morphology of our films with characteristic features of the cells themselves. Our hypothesis is that by introducing systematic roughness at biologically relevant length scales, it will be possible to tune the interaction of the modified substrate with the living tissue. Posters from the Albany-Cornell group presented at the 27th Neural Prosthesis conference also provide support for this position. They demonstrated selective adhesion of cells with patterned, rough silicon surfaces.

Reliable, reproducible results of the impedance of the coated probes as function of thickness and morphology are now being obtained. In these experiments, we monitor the impedance change as a function of frequency from 1 Hz to 1 Mhz. The results to date indicate that thin, bioactive coatings of protein polymer do not provide a significant increase in the impedance of the probe. One experimental problem which confounded our earlier efforts was the finding that the formic acid solvent used to process the protein polymer was attacking the epoxy used to mount the probes in the package. This was overcome by using a silicone-based sealant in place of the epoxy.

Plans:

We have ordered two conical indentors for the Nanoindentor mechanical testing apparatus which will enable us to obtain information about the modulus and strength of the protein thin films deposited on silicon substrates. Both indentors have 90 degree included angles with hemispherical tips, one has a radius of 1 micron, and the second a radius of 10 microns.

We are continuing experiments to elucidate the electrical properties of the protein films deposited on probes. We expect that there should be significant differences in impedance between continuous and discontinous films, particularly for thicker films.

We have now coated seive probes with SLPL for Dr. Allen Mensinger of Washington University Medical School in St. Louis. In the first run 5 probes were coated, and in the latest run 11 probes were coated. At the Neural Prosthesis meeting, we were contacted by other groups interested in coatings including Daryl Kipke of Arizona State University and James Weiss of the Jet Propulsion Laboratory. We are actively initiating collaborations with these investigators in order to obtain additional information about the biological response of our coatings.

We are developing our previously reported schemes for surface patterning, with particular interest in tailoring the topology to enhance cellular adhesion.

2. Bioactivity of Protein Polymer Films *in vitro*

Progress:

The *in vitro* studies of the biological efficacy of electrodeposited films were completed, and the results were compared to those of dip coated films. It was found that the cell spreading efficiency, measured as the percent of cells spread as a function of percent surface coverage, was not substantially different for the electrospun films. This indicates that to a first approximation the biological response of the SLPF is not a function of morphology. This is evidently due to the fact the large number of fibronectin binding sites present in the backbone of the molecule make it efficient for cells to find an appropriate site to initiate an adhesive interaction.

Plans:

We are currently pursuing bioactivity experiments of coatings containing drugs and other molecules with specific functionality. We have already found that coatings of SLPF containing kanamycin are effective in limiting the growth of bacteria. In this study, the coating was prepared with 75% weight percent SLPF and 25% kanamycin.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress:

Polypropylene suture (~50 micron diameter) was coated with the following materials and implanted in the Guinea Pig CNS:

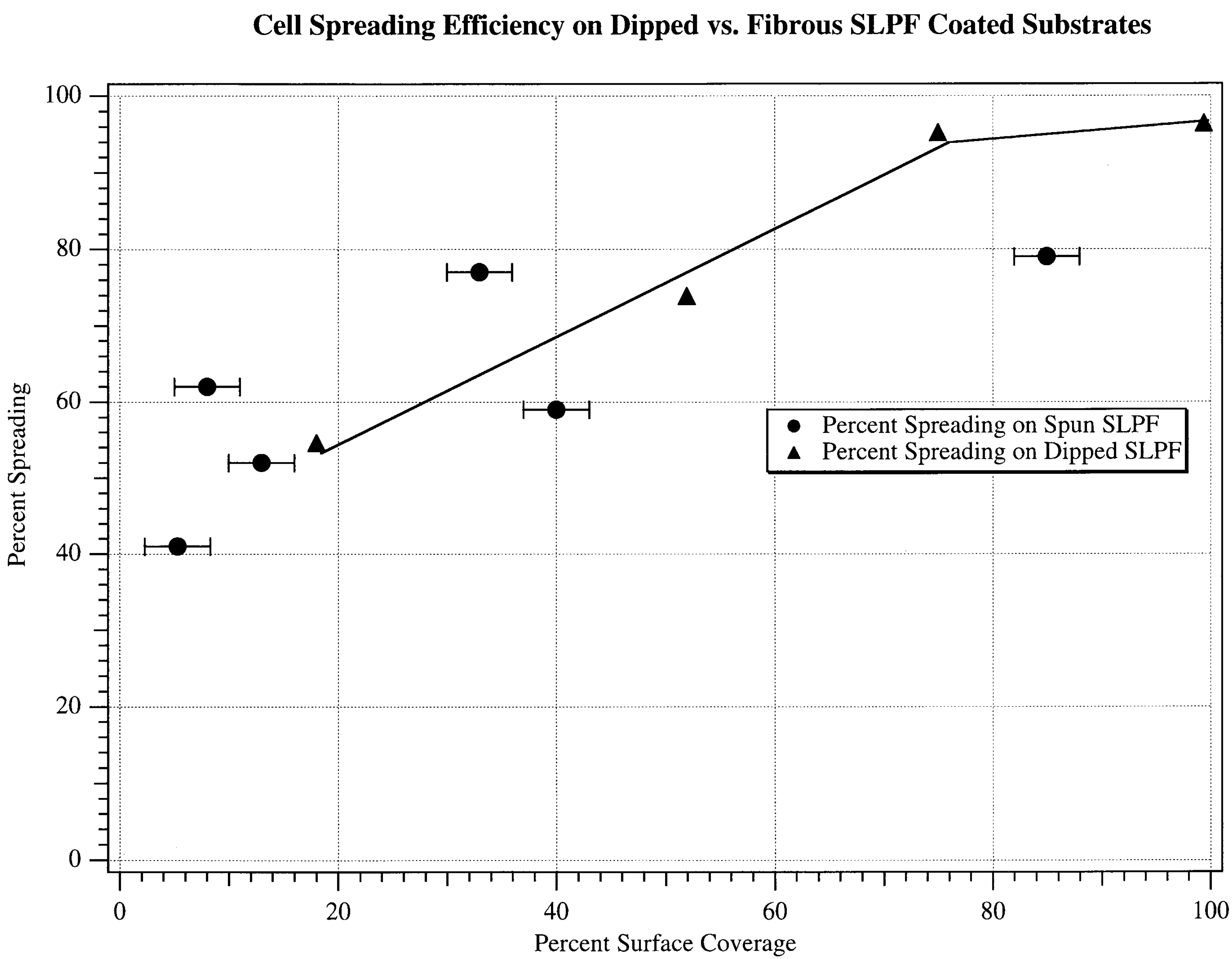
1. no coating (control)
2. SLPF coated
3. SLPL coated
4. SLPF/Schwann cells
5. SLPF coated and exposed to CSF
6. SELP coated

Nine month samples were removed and examined histologically by optical microscopy. Copies of the histological results are included with this report.

4. External Communications

A talk on "Surface Modification for Biocompatibility" was presented at the 27th Annual Neural Prosthetics Workshop at the National Institute of Health in Bethesda, MD.

The proofs for the review paper on "Processing and Characterization of Protein Polymers" were received and returned.



10/31/96
CJ Buckleo

SLPh coated seive probe
from A. Meusinger

10/31/96
CJ Buchko

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12 September, 1996

Joseph Cappello, Ph.D.
Director of Polymer Research
Protein Polymer Technologies, Inc
10655 Sorrento Valley Road, First Floor
San Diego, CA 92121-1624

Dear Joe,

Enclosed is a copy of the report on the first phase of work designed to examine the tissue reaction of protein polymers in adult CNS. As you know, work on this project is ongoing and we hope to have additional data on the nine month response very soon. Thanks again for your help in making this project possible.

Yours sincerely,

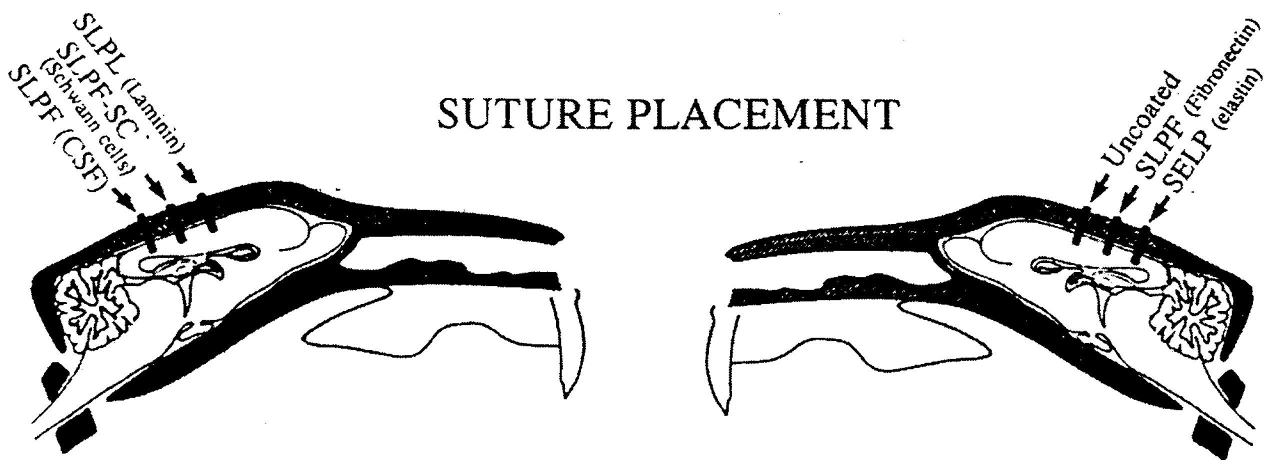
Sue

K. Sue O'Shea, Ph.D.
Associate Professor

This is a complete copy of the report sent to Joseph Cappello in September, 1996. Since that time we have made observations on the nine month implants (Figures 3 and 4, appended). There is remarkably little response of the CNS to implantation at this time. Current investigations are in progress to examine the response of glial cells at a distance to the implant site using immunohistochemical localization of both GFAP and also of microglial cells.

CNS response to biopolymer coated suture

The long term goals of these studies are to improve stability of probes chronically implanted into adult CNS. To first determine local reaction of CNS tissue to the coatings themselves, adult guinea pigs were used. Sutures were implanted in this pattern:



Observations of tissue response have been made three months after implantation into adult guinea pig cortex of 6-0 polypropylene suture (Figure A,B), spin coated with SLPF (C,D), SLPL (E,F), or SELP (I,J). SLPF suture was also coated with neonatal mouse Schwann cells by 24h co-culture in roller bottles (G,H); additional SLPF coated suture was dipped in bovine CSF immediately prior to implantation (K,L).

Guinea pigs were anesthetized with chloral hydrate, sutures implanted through burr holes and animals sacrificed after 3 weeks or 3 months. An additional series of three animals was implanted with coated suture and were sacrificed in August, 1996, for nine month observations. Animals were anesthetized with chloral hydrate, perfused transcardially with PBS followed by 4% paraformaldehyde, (or 2% PF; 1% GA). Brains were stored in fixative, transferred to buffer followed by step-wise dehydration and embedment in araldite. An initial series of brains was embedded in methycrylate (for immunohistochemistry), but we determined that methycrylate was too soft to allow sectioning of the suture, and a resin with similar hardness to suture, araldite, was employed to obtain sections of suture and surrounding brain tissue.

To date it has been possible to carefully examine the reaction to implantation in five 3 month animals and in two three week animals. At three months, we have observed slight asymmetry in tissue reaction to implantation which appears to be due to the coating itself. The coatings do not increase reaction, in fact it appears that compared with uncoated suture, polymer coating decreases reaction of the CNS to implantation.

Histologically, we have observed a mild reaction of glial cells surrounding the implant. There is a re-orientation of glia immediately surrounding the suture, which extends from three-five cell diameters. Glial nuclei are flattened and elongate, and do not appear to be reactive. In one occasion we observed a single microglial cell in the region. Just outside the rim of glial cells, neuronal cell bodies and nuclei appear morphologically normal, with no evidence of chromatin alterations. These observations were confirmed in consultation with C.J. D'Amato of the University of Michigan Neuropathology staff.

We have carried out a limited number of observations at the TEM level (one per group), and in each case, there is a flattened rim of glial cells adherent to the implant. There is a slight variation in the number of strata which surround the implant, but variation between animals is greater than that between coatings. Based solely on morphological appearance, it is not possible to exclude fibroblasts as the source of cells rimming the suture, nor is it possible to determine conclusively that there is not a glial (or neuronal) reaction at some distance from the implant. In the next phase of the work, cortex will be processed for immunohistochemical localization of glial fibrillary protein to determine the extent of glial reaction to implantation.

This work was funded by NIH contract, "Surface Modification for Biocompatibility", NO1-NS-5-2322 and polymer provided by Protein Polymer Technologies, Inc.

Richard A. Altschuler
Professor

K Sue O'Shea

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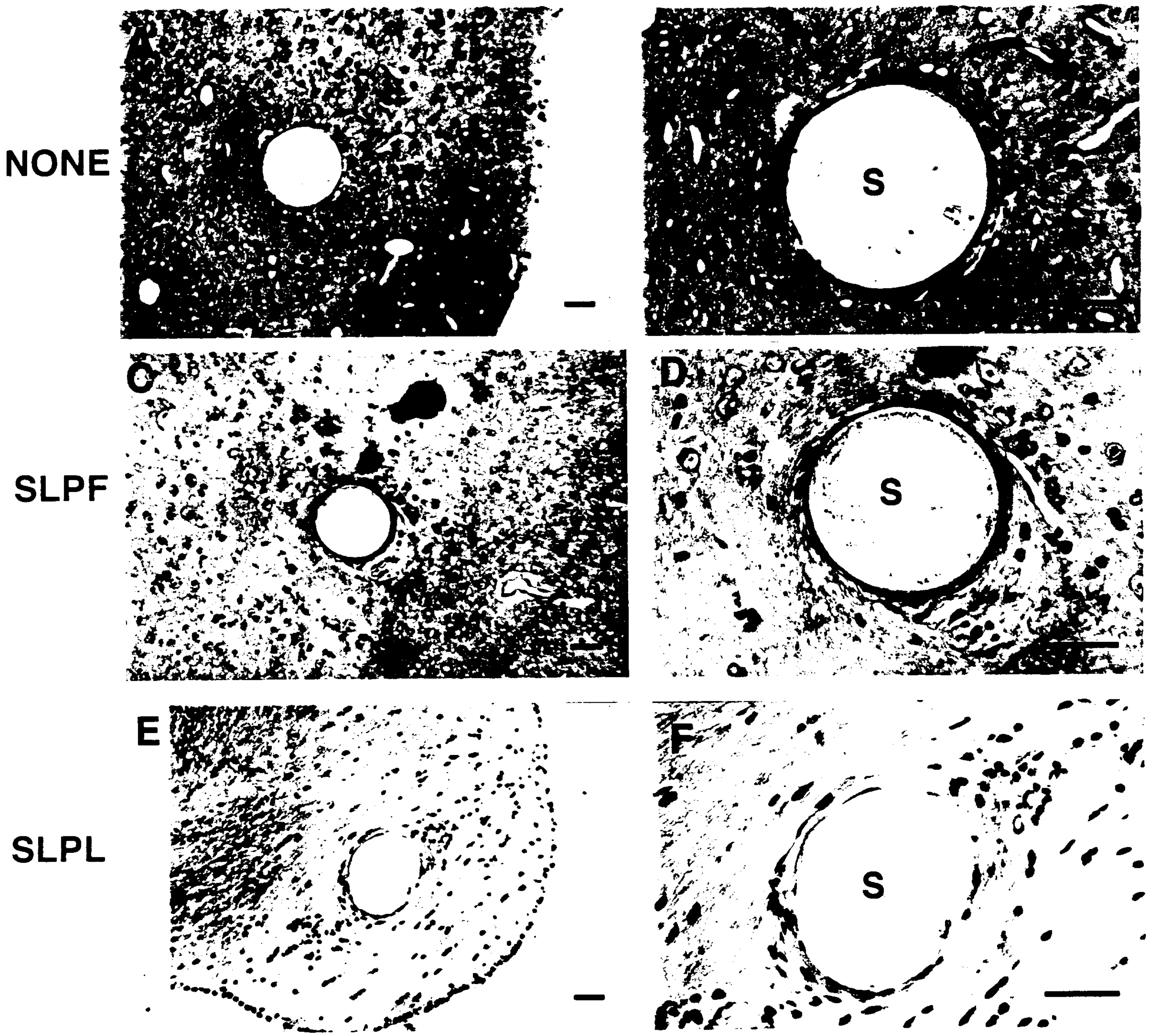
NONE
SLPF
SLPL
C
D
E
F
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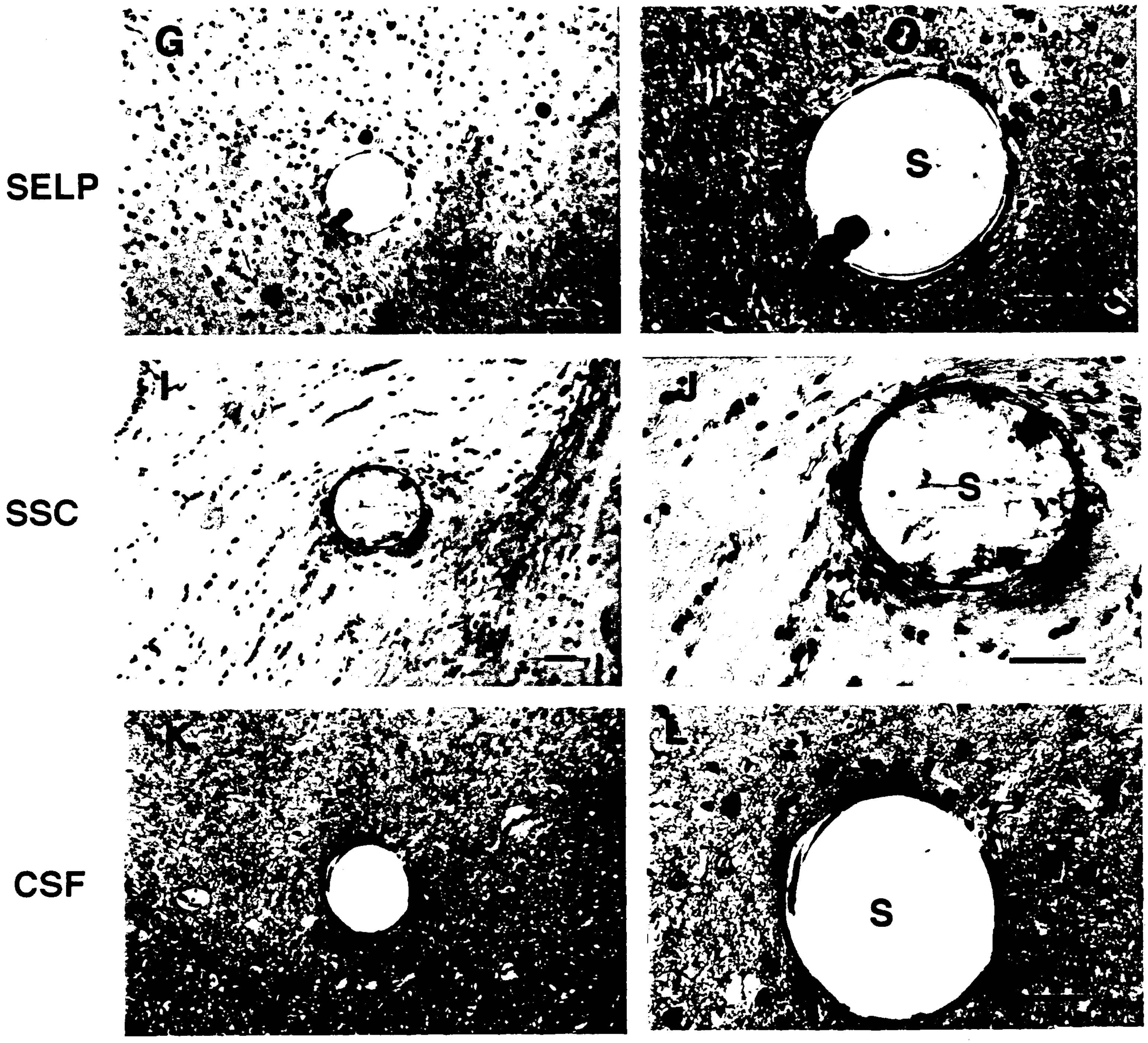
S

2

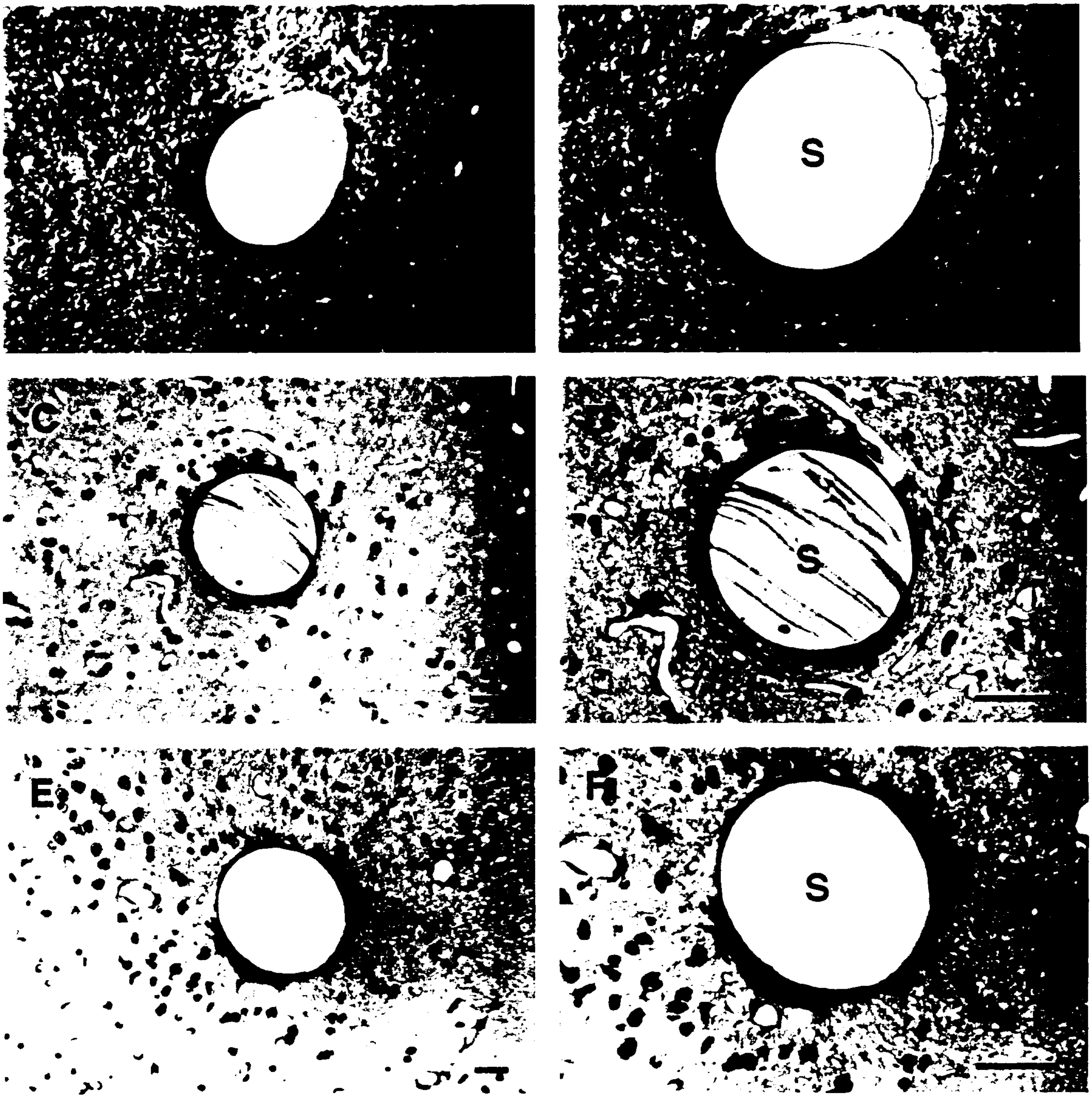


3

NONE

SLPF

SLPL



4

SELP

SSC

CSF